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The cytochrome bc_1 complex and the evolution of membrane bioenergetics

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We have previously argued that the use of sodium ion gradient for ATP synthesis is the ancestral modality of membrane bioenergetics and that the Last Universal Cellular Ancestor (LUCA) was unlikely to have proton-dependent energetics [1–4].

The evolutionary primacy of the sodium-dependent membrane bioenergetics contradicts the common belief that the LUCA possessed several proton pumps, such as cytochrome oxidase and quinol: cytochrome c oxidoreductase, which are widespread among bacteria and archaea. To address this conundrum, we analyzed the phylogeny of the quinol: cytochrome c oxidoreductases (cytochrome bc_1 complexes) and showed that the phylogenetic tree of quinol: cytochrome c oxidoreductases did not follow the 16S rRNA tree. We suggest that the common ancestor of the quinol: cytochrome c oxidoreductases evolved within bacteria from a membrane quinone oxidoreductase analogous to the complex II, perhaps in response to the emergence of chlorophyll-based photosynthesis. Different archaeal phyla seem to have acquired different types of quinol: cytochrome c oxidoreductases from bacteria by lateral gene transfer on several independent occasions. A similar scenario has been proposed for the evolution of the cytochrome oxidases [5].

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Effects of ascochlorin on the yeast *Candida albicans*

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Ascochlorin and ascofuranone are antibiotics produced by the phytopathogenic fungus *Ascochyta viciae*, and both have closely related prenylphenol structures like ubiquinol. Ascofuranone specifically inhibits trypanosome alternative oxidase, and is considered to be a promising candidate as a chemotherapeutic agent against African trypanosomiasis. On the other hand, ascochlorin specifically acts at Q_i and Q_o sites of cytochrome bc_1 complex [1] to inhibit the electron transport. We report the effects of ascochlorin on the pathogenic yeast *Candida albicans*.

Ascochlorin also acted on the cytochrome bc_1 complex to inhibit the cyanide-sensitive respiration of *C. albicans* as well as antimycin A, stigmatellin, and myxothiazol. Further, ascochlorin induces the expression of nuclear-encoded cyanide-resistant alternative oxidase gene, and inhibits the alternative oxidase activity. However, the inhibitory effect was weaker (about 20%) than that of ascofuranone. Interestingly, the amino acid residues of alternative oxidase involved in the inhibitor (or ubiquinol)-binding are completely different from those of cytochrome bc_1 complex. Therefore, ascochlorin inhibits both respiratory electron transports (cyanide-sensitive and cyanide-resistant) in this yeast.

To examine the effects of respiratory inhibitors, *C. albicans* was cultivated using a variety of carbon sources (glucose, acetate, ethanol et al.) at several concentrations. Under all conditions tested, among the respiratory inhibitors, antimycin A showed the most potent inhibition on the aerobic growth, which was slightly increased by the combined addition of ascofuranone. Ascochlorin had a little bit lesser growth inhibition, which was increased in the presence of ascofuranone.

These results suggest that antimycin A induces little alternative oxidase gene expression in spite of its helpful role in the aerobic growth. Although ascochlorin was able to inhibit alternative oxidase activity, this antibiotic induces alternative oxidase gene expression to transport electrons to oxygen, thereby making a contribution to the aerobic energy metabolism in *C. albicans*.

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Inter- and intra-monomeric communication in the cytochrome bc_1 complex as studied by molecular dynamics simulations

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The cytochrome bc_1 complex (bc_1) acts as a homodimeric proton translocase, as reviewed in [1]. It oxidizes ubiquinol molecules (Q_P) in the catalytic centers *P* via bifurcated reaction and reduces two distinct substrates: the FeS cluster of the Rieske protein and, via the two hemes *b*, the ubiquinone molecule located in another quinone binding site *N* (Q_N), (according to the Mitchell's *Q*-cycle [2]).

Earlier we have shown that the reaction in the Q_P site is kinetically coupled with the quinone reduction in the Q_N site, so that the relocation of the FeS domain towards its electron acceptor cytochrome c_1 happens only after the ubiquinol formation in the center *N* [3–5]. However, the mechanism of the suggested intra-monomer and

inter-monomer coupling within the bc_1 dimer [4, 6] has remained elusive.

Here we have used large-scale molecular dynamics (MD) simulations for tracking the communications within the bc_1 of *Rhodobacter sphaeroides*. The energy correlation analysis [7] revealed the possible pathways for transmembrane propagation of information about the redox state of the ubiqinone molecule in the Q_N site. Non-equilibrium MD simulations of the bc_1 with ubiquinone or ubisemiquinone in the Q_N site, respectively, revealed the possible implication of helix *E* and the *ef*-loop of cytochrome *b* in the intra-monomeric transmembrane coupling. The MD simulations with occupied and unoccupied Q_P site showed a possible pathway for the inter-monomeric communication between the two Q_P sites of a bc_1 dimer.

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12P4

In vivo accumulation of coenzyme Q biosynthetic intermediates and aminated analogs in the yeast *Saccharomyces cerevisiae*

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Coenzyme Q (ubiquinone or Q) is a redox-active lipid essential for electron and proton transport in the mitochondrial respiratory chain. Q is also important in the mitochondrial inner membrane because it serves as an antioxidant and it modulates the function of the mitochondrial membrane transition pore [1]. Most Coq proteins which participate in Q biosynthesis are present in a high molecular mass multi-subunit complex in *Saccharomyces cerevisiae*. The absence of a single Coq polypeptide from the complex causes a drastic diminution of the steady state levels of some Coq proteins. In consequence, only an early intermediate of the Q biosynthetic pathway accumulates in Δ*coq1*–Δ*coq9* strains [2].

We report that overexpression of the protein kinase Coq8 restores the steady state levels of the Coq proteins in most Δ*coq* strains. The stabilization of the Coq polypeptides leads to the accumulation of Q biosynthetic intermediates [3]. These intermediates are likely not competent to transfer electrons in the respiratory chain because the strains are not capable of growing on non-fermentable carbon sources. However, we suggest that some of these Q biosynthetic intermediates have an antioxidant activity and are therefore able to redox-cycle *in vivo* since they diminish the sensitivity of the accumulating strains to poly-unsaturated fatty acids.

4-hydroxybenzoic acid is the precursor of the aromatic ring of Q. We demonstrate that analogs of 4-hydroxybenzoic acid can be used *in vivo* to either promote the synthesis of analogs of coenzyme Q [4] or to bypass a deficient Q biosynthetic step. In this later case, the use of vanillic acid allowed the restoration of Q biosynthesis and respiration in cells deficient for the monooxygenase Coq6 [5].

Our data illustrate the possibility to generate Q analogs *in vivo* which offers the opportunity to study the structural requirements of Q for its different cellular functions.

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12P5

How to inhibit bc_1 complex with antimycin A?

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Using a stochastic simulation without any other hypotheses, we have demonstrated the natural emergence of the Mitchell Q-cycle in the functioning of the bc_1 complex, with few short-circuits and a very low occupancy of the reactive semiquinone species in the Q_o site [1]. However, this simple model fails to explain both the inhibition by antimycin of the bc_1 complex and the accompanying increase in ROS production.

To obtain inhibition of electron transfer to the high potential chain in the presence of antimycin, it is necessary to block the electron transfer from the reduced haem b_L to the semiquinone in the Q_o site (short-circuit or bypass of type 2) [2]. Incorporating this constraint in our stochastic model, we obtain a sigmoid inhibition curve due to the fact that when only one antimycin is bound per bc_1 dimer, the electron of the inhibited monomer systematically crosses the dimer interface to reduce a quinone or a semiquinone species in the other (free) Q_i site (b_L–b_L path). Because this step is not limiting, the activity is unchanged (compared to the activity of the free dimer). Interestingly, this b_L–b_L pathway is almost exclusively taken in this half-bound antimycin dimmer. In the free dimer, the natural faster pathway is b_L–b_H on the same monomer (at least in the absence of $\Delta\mu H^+$). The addition of the assumption of half-of-the-sites reactivity to the previous hypothesis leads to a transient activation in the antimycin titration curve preceding a quasi-complete inhibition at antimycin saturation.

In accordance with the chemistry of quinone, we have examined the possibility that the return of the electron from the reduced haem b_L to the semiquinone in Q_o could be blocked if we take into account the protons transfer and release from the Q_o site accompanying the electron transfer. However preliminary simulations show that it is not